The Effect of Protein Particle Size Reduction on the Physical Properties of CO$_2$-Precipitated Casein Films

KIRSTEN L. DANGARAN, PETER COOKE, AND PEGGY M. TOMASULA

ABSTRACT: Casein precipitated with high pressure-CO$_2$ (CO$_2$CAS) has unique properties compared to commercial acid-precipitated casein. CO$_2$CAS is less water-soluble and films made from it are less susceptible to high humidity environments; however, the films are also opaque and hazy. The appearance of CO$_2$CAS films is important especially if applied as a food coating. To improve the appearance properties, the particle size of CO$_2$CAS film plasticized with glycerol was reduced. The effect of protein particle size reduction on tensile properties, water vapor permeability (WVP), and gloss was studied using ASTM methodology. As particle size of the CO$_2$CAS was reduced from 126 μm to 111 μm, tensile strength and modulus of the films increased, while WVP decreased. With the same particle reduction, gloss increased from 55.3 gloss units on average to 73 gloss units, but films were still hazy. With a particle size less than 86 μm, CO$_2$CAS films were glossy and transparent, however, tensile strength decreased and WVP increased. Depending on desired application, the properties of CO$_2$CAS films can be optimized by changing particle size.
    Keywords: particle size, permeability, edible films, tensile properties, CO$_2$-precipitated casein

E: Food Engineering & Physical Properties

Introduction

Edible films and coatings serve an important role in the protection and preservation of food. Acting as barriers to biological or chemical damage, films and coatings can extend the shelf-life of a product. Much work has been done on edible films and coatings made from agricultural materials such as soy protein, caseinate, whey protein, starch, and corn zein (Brandenberg and others 1993; Gennadios and others 1994; Psomiadou and others 1996; Cuq and others 1998; Cagri and others 2004). These materials have been found to have gas barrier or tensile properties similar to synthetic packaging materials like polyethylene, PVC, or EVOH. However, most films made from these proteins or polysaccharides are hydrophilic, making them sensitive to high moisture environments and causing them to become tacky and to swell.

Tomasula and others (1995) have developed a process to isolate casein from milk using high-pressure CO$_2$. Under elevated pressure and temperature, the solubilized CO$_2$ creates an acidic environment, which causes the casein to aggregate and precipitate. The CO$_2$-precipitated casein (CO$_2$CAS) is less soluble in water than calcium or sodium caseinate, and the micellar structure remains intact during the high-pressure precipitation process. Moreover, CO$_2$CAS creates films that are better moisture barriers and are less susceptible to high relative humidity (RH) than other previously mentioned protein- and starch-based films with the exception of corn zein films, which are hydrophobic. However, because films are made from dispersions of CO$_2$-casein in water, they are hazy and opaque and have rough surface topography (Tomasula and others 1998).

For many film or coating applications, a transparent or glossy appearance is desired by consumers (Lee and others 2002). CO$_2$CAS films have unique and desirable properties, but the issue of haze must be addressed. The improvements to the appearance should be made with little or no deterioration of the water vapor barrier and good tensile properties of the films. Large particles scatter light reflecting off a film's surface, reducing gloss and giving it a hazy appearance (Hunter 1987). We hypothesized that decreasing the particle size of the CO$_2$-casein in the dispersion would decrease haze and improve gloss.

As particle size is decreased, the appearance properties of the CO$_2$-casein films may not be the only properties affected. Changes to the size of the CO$_2$-casein particles will likely affect the tensile properties. More particles will be created and total surface area of the protein particles will also increase, creating more opportunities for protein–protein interactions at particle interfaces. The strength, stiffness, and flexibility of protein films is correlated to the extent of hydrophobic and electrostatic interactions between colloidal particles. It is hypothesized that more plasticizer will be needed to increase flexibility of the CO$_2$CAS films, as particle size is decreased because of increased protein–protein interactions. Water vapor permeability (WVP) is the measure of moisture migration through the film. It can be affected by changing the solubility of the water in the film system or by changing the diffusion coefficient of the water in the film. In composite film systems of hydrocolloids and lipids, decreasing particle size of the lipid improved the moisture barrier properties (McHugh and Krochta 1994; Perez-Gago and Krochta 2001). In parallel, it is hypothesized that decreasing particle size of the insoluble CO$_2$-casein will create a more tortuous path for water molecules to travel through the film and decrease water vapor transmission rates. An assumption is that the diffusing water will move through the continuous phase of the film and not through the dispersed CO$_2$CAS particles (Rogers 1985).

In this study, films were made from calcium caseinate (CaCAS) and CO_2CAS and were plasticized with glycerol. The plasticizer content was varied along with particle size of CO_2CAS. Protein particle size was affected by changing the shear treatment of film-forming dispersions. The objective of this study was to determine the effect of CO_2-casein particle size on the WVP, appearance, and tensile properties of CO_2CAS films. The results were compared to those of CaCAS films, which are transparent films and are being used as an appearance standard.

Materials and Methods

Materials

Films were made from either calcium caseinate (Alanate 310, New Zealand Milk Products, Inc., Santa Rosa, Calif., U.S.A.) or from high-pressure CO_2-precipitated casein. The CO_2-casein was made following the procedure of Tomasula and others (1995), previously mentioned. Skim milk (6 °C) purchased from the local supermarket (500 g) was placed in a 1000-mL Parr batch reactor (model 4521 316SS, Parr Instrument Co., Moline, IL), and the reactor was sealed. The milk was heated to 40 °C. CO_2 was introduced to the batch reactor over a 3-min period until pressure reached 800 psi. The milk was held under these conditions for 5 min to precipitate the casein protein. To depressurize the system, an outlet was opened that allow the pressurized liquid whey to flow out of the batch reactor. The whey was filtered as it left the reactor to minimize loss of precipitated casein. The casein was collected and weighed, and the moisture content was determined. The casein was washed with distilled–deionized water to bring the lactose content to less than 0.5%. After washing, the casein was freeze-dried (Freezemobile 12 XL, VirTis, Gardiner, N.Y., U.S.A.), ground (micronizer, Warring Laboratory Science, Torrington, Conn., U.S.A.) and sieved (300 μm and 250 μm, The Tyler Co., Cleveland, Ohio, U.S.A.). CO_2-casein particles were initially ≤250 μm. Protein solutions or dispersion were made with distilled-deionized water (Barnstead International, Dubuque, Iowa, U.S.A.).

Film solutions/dispersions

CaCAS solutions were made by dissolving the protein in water. The caseinate solutions were 10% w/w. Calcium caseinate solubilizes but initially clumps when introduced to water. To promote hydration, break up clumps, and hasten the solution-making process, a hand-held immersion blender (Braun, The Gillette Company, Boston, Mass., U.S.A.) was used for 2 min. The blender mixed at 12000 rpm. The calcium caseinate was allowed to continue to dissolve for 30 min prior to the addition of the plasticizer. The solutions were plasticized with glycerol (Sigma-Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) at a level that the final dried films were either 10%, 20%, or 30% plasticizer by mass. The solutions were degassed under vacuum with a direct drive pump (Precision Scientific, Winchester, Va., U.S.A.). On the basis of total solids of each solution, 1.5 g of solids were cast in 100 × 15-mm polystyrene Petri dishes

(Fisherbrand, Fisher Scientific, Fairlawn, N.J., U.S.A.). Films were allowed to dry overnight at ambient conditions. Films were then peeled and conditioned in an environmental chamber kept at 53% RH with a saturated magnesium nitrate solution (Sigma-Aldrich, Milwaukee, Wis., U.S.A.) for 2 d.

CO_2CAS dispersions were made using an Ultra-Turrax model T25 (IKA Works Inc., Wilmington, N.C., U.S.A.). Compared to the hand blender, the Ultra-Turrax produced higher and variable shear treatments that were needed to disperse the CO_2-casein. The 10% wt/wt protein dispersions were mixed for 2 min using the 6500 rpm (UT1), 13500 rpm (UT3), or 24000 rpm (UT6) setting. For each setting, dispersions were plasticized at the same levels as the CaCAS solutions—10%, 20%, or 30%. Films were cast, dried, and conditioned as described above. Table 1 shows a list and description of all the variables and variable codes used in this study.

Water vapor permeability measurements

After conditioning, films were tested for WVP following the ASTM methodology (ASTM 1995). Film thickness was measured (micrometer, model LG3602-02-04, Ames, Waltham, Mass., U.S.A.) five times for each sample, and an average was calculated. Films were sealed in Plexiglass cups with an exposed area of 20.27 cm² . Distilled water was used to create a 100% RH environment on the inside of the cup. The air gap between the film and the surface of the water was 0.6 cm. Cups were put in a chamber with air circulation and with a targeted RH of 0% (Drierite). Actual RH in the chamber was measured to be 8% ± 3% RH with a hygrometer/thermometer pen with memory (Traceable Fisherbrand, Fisher Scientific, Fairlawn, N.J., U.S.A.). Room temperature was 25 °C ± 2 °C. The initial weight of each cup was taken, and then the weight was measured over a 24-h period at intervals that were at least 3 h long. WVTR was calculated from the slope of the linear regression (Excel, Microsoft, Redmond, Wash., U.S.A.) of weight loss versus time data. Permeance was calculated using the correction method for hydrophilic films described by McHugh and others (1993).

$$Permeance = \frac{WVTR}{p_2 - p_3},$$

where p_2 is the corrected partial pressure at the inner surface of the film, and p_3 is the partial pressure at the outer film surface. WVP was determined by multiplying permeance by average film thickness.

Tensile properties measurements

After conditioning for 48 h at 53% RH, the films were cut into 5 × 35 mm strips. Tensile properties were measured following the standardized method for plastic sheeting films (ASTM 2001). To allow comparison to results from previous studies of CO_2CAS film properties (Tomasula and others 1998), a grip head speed of 5 mm/min was used. Initial grip separation was 25 mm. Tensile strength, elastic modulus, and percent elongation at break were calculated. These properties were measured using an Instron Universal Testing Machine (Instron Corp., Canton, Mass. U.S.A.). For each replicate, five strips of film were tested.

Table 1 — Variable codes and descriptions

Protein	Mixer	Mixer setting[a]	Variable code		
			10% Glycerol	20% Glycerol	30% Glycerol
Calcium caseinate	Hand blender	12000 RPM	10CA	20CA	30CA
CO₂-casein	Ultra-Turrax	UT1 (6500 RPM)	10UT1	20UT1	30UT1
CO₂-casein	Ultra-Turrax	UT3 (13500 RPM)	10UT3	20UT3	30UT3
CO₂-casein	Ultra-Turrax	UT6 (24500 RPM)	10UT6	20UT6	30UT6

[a]All settings were mixed for 2 min.

Appearance properties

Gloss and haze of the flat films were measured using a Micro-Tri-Gloss meter (Tricor Systems Inc., Elgin, Ill., U.S.A.). The instrument measures specular reflection of the surface of the films when light is incident at either a 20°, 60°, or 85° angle to the normal. The instrument is calibrated with a black and flat mirror with a refractive index of 1.567. Films were placed on a matte, flat and black surface that has negligible measured reflectance. Gloss of the films was measured at the 20° and 60° angles according to the standardized method for flat surfaces (ASTM 1989). For gloss results of this study, the values measured at the 60° angle only are reported. For high-gloss surfaces, haze can be calculated by subtracting the gloss value at 60° from the gloss value at 20° according to the standardized method for reflection haze (ASTM 1999). The larger the difference between the measurements is, the hazier the sample. A minimum gloss value (gloss units) of 70 with a 60° angle of incidence is needed for a surface to be considered high gloss.
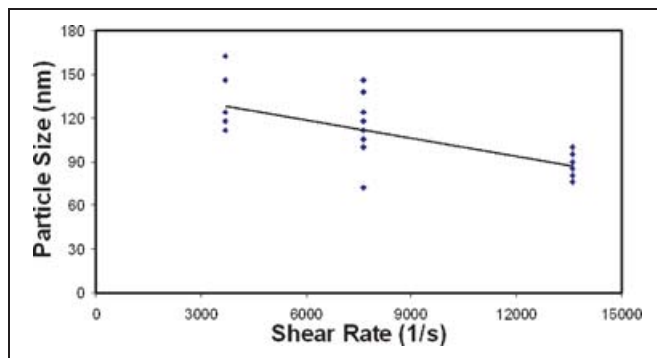
Particle size analysis

The particle size distribution of the CO_2CAS aqueous dispersions was measured using an AccuSizer Model 780 (Particle Sizing Systems, Inc., Santa Barbara, Calif., U.S.A.). The samples were injected into the AccuSizer equipped with an autodilution system. Visual inspection of the CO_2CAS dispersions found an observable decrease in particle size going from the UT1 to UT6 treatments. However, for all CO_2CAS protein dispersion, the particle size distribution was large, and it was difficult to compare samples on the basis of the entire distribution. Therefore, changes in particle size for the dispersions were verified by correlating the minimum size of the particles in the top 10% of the distribution to shear rate. This procedure was similar to that used by Driscoll (2004) and Driscoll and others (2002) to investigate changes in lipid particle size in parenteral emulsions. They monitored increasing lipid size by invoking the "large-diameter tail" method.

Microscopic imaging

SEM analysis. Strips of the film, approx. 1 × 2.5 cm, were excised with a stainless steel razor blade, and each strip was immersed into 20 mL of 2.5% glutaraldehyde-0.1M imidazole buffer HCl at pH 7.2 for several hours to cross-link the protein. Following this initial step, the strips were washed in buffer, dehydrated in a graded series of ethanol solutions (50%, 80%, absolute), and frozen in liquid Nitrogen. The frozen strips were fractured manually using fine forceps, across the longitudinal axis, and the fragments were quickly thawed into absolute ethanol. Next, the fractured pieces were critical point dried from liquid CO_2, and the dry pieces were mounted onto aluminum specimen stubs with Duco Cement (ITW Performance Polymers, Riviera, Fla., U.S.A.) with the fractured faces up. Finally, the samples were sputter coated with a thin layer of gold and examined in a Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, Oreg., U.S.A.), operated in the high vacuum and secondary electron imaging mode. Digital images were collected at magnifications of 1000× and 10000×. Only the 10000× images are presented.

Stereofluorescence. Optical imaging of the strips was done with a model MZ FLIII stereofluorescence microscope, equipped with a DC100 charge-coupled device camera (Leica Microsystems, Bannockburn, Ill., U.S.A.) and transmitted illumination from a 150 W halogen lamp in an Intralux 5000-1 lamphouse (Volpi Manufacturing, Auburn, N.Y., U.S.A.).

Figure 1 — Pearson correlation analysis of minimum particle size of the top 10% largest particles in the CO₂CAS dispersions and shear rate

Statistical analysis

Three replicates were made for each variable. A two-factor, complete block design was used for this study. ANOVA was performed using SAS (SAS Institute Inc., Cary, N.C., U.S.A.). Correlation calculations were done with Excel (Microsoft, Redmond, Wash., U.S.A.).

Results and Discussion

Particle size

Figure 1 shows the change in minimum particle size for the top 10% largest particles in each dispersion population versus shear rate. Shear rate was calculated from the diameter of the rotor of the Ultra Turrax (17 mm), the rotation rate of the Ultra Turrax, and the gap between the rotor and stator (0.5 mm).

$$\text{Shear Rate} = \frac{(\text{Diameter of Rotor})(\text{rpm})}{(\text{Gap})(60\,\text{s}/\min)}$$

Particle size analysis showed a significant negative correlation (Pearson coefficient $R = -0.730$, $P < 0.001$) between shear treatments (rpm of Ultra-Turrax) of the CO_2CAS solutions and particle size. As shear stress increased in the form of increased rotations per minute of the homogenizer, the particle size of the dispersions decreased. For the top 10% of the particle distributions, on average, particle size decreased from 126.4 μm to 111.8 μm to 85.9 μm for the UT1, UT3, and UT6 treatments, respectively. The UT6 average particle size was significantly smaller than the UT1 and UT3 dispersions, based on ANOVA. As shear treatment increased, the scatter of particle size in the largest 10% of the population decreased, indicating increasing homogeneity.

Gloss properties

Appearance properties of the CO_2CAS films were significantly affected by decreasing particle size. As a reference, the gloss and haze

Table 2 — Average gloss values for calcium caseinate and CO₂-precipitated films plasticized with various levels of glycerol

	Average gloss value of films		
Protein and treatment	10% Glycerol	20% Glycerol	30% Glycerol
Ca Caseinate — hand blender	130.5[a]	149.3[a]	147.0[a]
CO₂CAS-UT6 (24000 rpm)	110.6[b]	118.1[b]	119.7[b]
CO₂CAS-UT3 (13500 rpm)	77.7[c]	79.2[c]	57.7[c]
CO₂CAS-UT1 (6500 rpm)	68.1[c]	54.8[d]	36.8[d]

[a–d]For each column, letters indicate significant difference at the 95% confidence level.
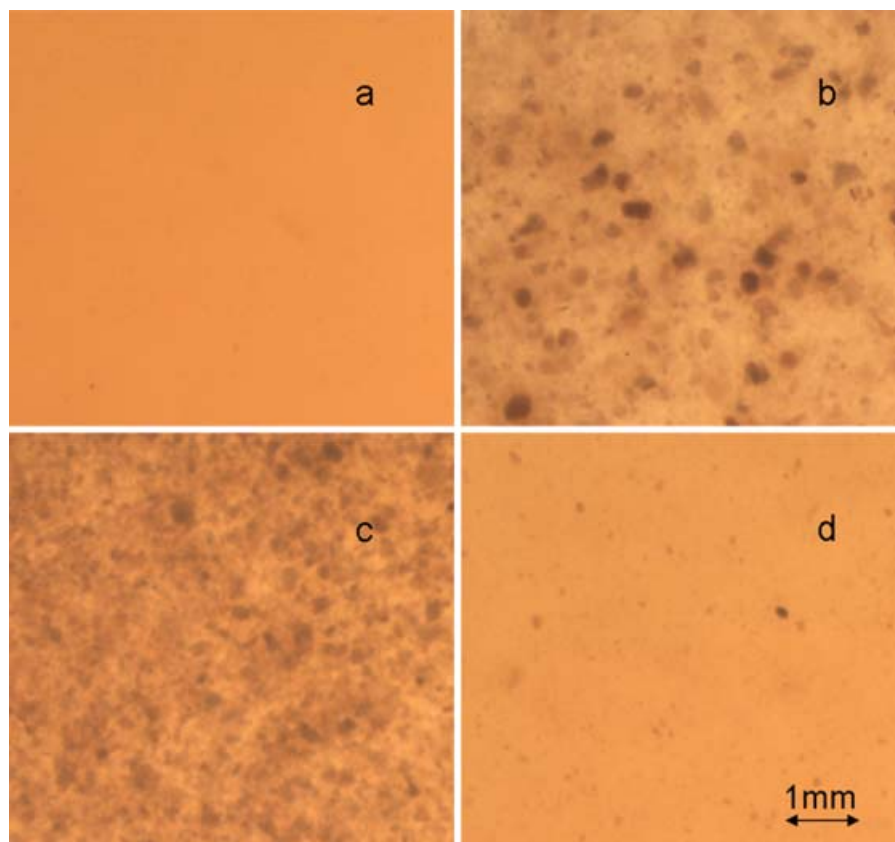
Figure 2 — Stereofluorescence images film made from calcium caseinate and CO$_2$-precipitated casein plasticized with glycerol: (a) 20CA; (b) 20UT1; (c) 20UT3; (d) 20UT6.
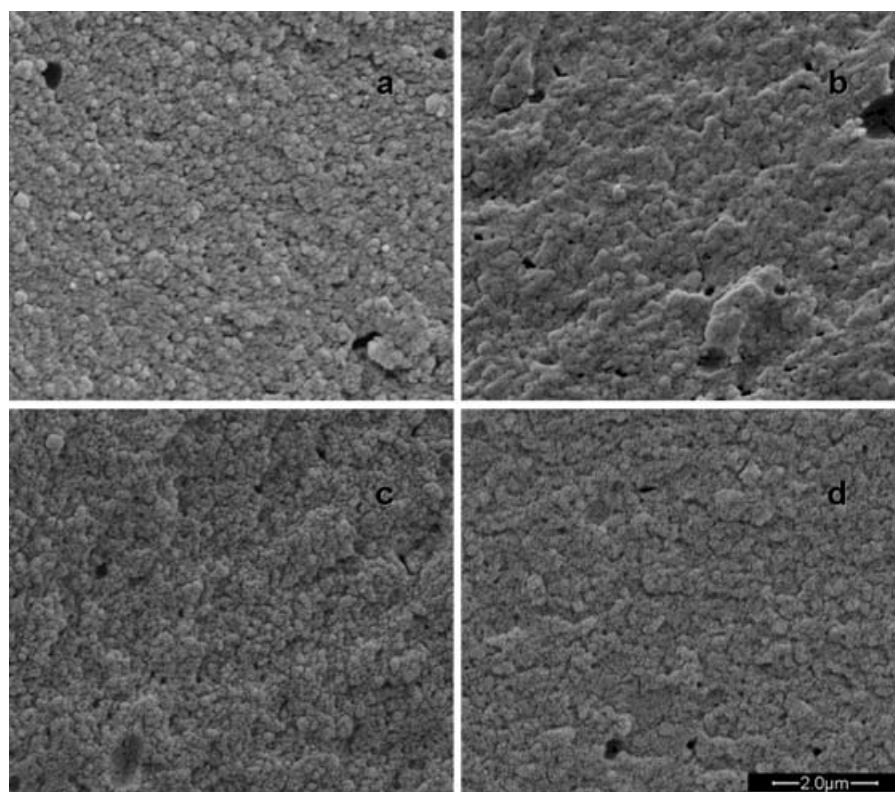
Figure 3 — SEM images film made from calcium caseinate and CO$_2$-precipitated casein plasticized with glycerol: (a) 20UT1; (b) 20UT3; (c) 20UT6; (d) 20CA.

E: Food Engineering & Physical Properties

of films made from CaCAS were measured, and the values were compared to those of the CO$_2$CAS films. CaCAS films were used because they are transparent and highly glossy, both desirable characteristics of edible films and coatings. Table 2 shows the gloss of all films measured with a 60° angle of incidence for the light source. For all glycerol levels, gloss followed the trend of CaCAS > UT6 > UT3 > UT1. Averaged over all glycerol levels, the CO$_2$CAS films with the largest particle size (UT1) would be considered semigloss surfaces (that is, gloss < 70), while all others were high-gloss surfaces. Therefore, the UT1 samples were not analyzed for haze according to the ASTM method used. The CaCAS films were transparent and highly glossy with an overall gloss value of 142.3. Reducing particle size of the CO$_2$CAS dispersions created films that much more closely resembled the CaCAS films. With the largest particle size, UT1 films were opaque and had an overall gloss value of 55.3. As particle size of the CO$_2$CAS decreased with shear treatment, gloss of the films significantly increased to 73.3 and 116.8 for the UT3 and UT6 samples, respectively. Also, haze was positively affected and reduced by decreasing particle size of the CO$_2$CAS. The CaCAS reference films had an overall haze value of 33.5. The UT3 samples were significantly hazier with an average value of 45.4. Decreasing particle size in the UT6 samples decreased haze to 40.7, a value not significantly different from the CaCAS film based on ANOVA.

Microscope imaging

Figure 2 and 3 show the stererofluorescence images and the SEM images of the CaCAS and CO$_2$CAS films, respectively. Comparing the stererfluorescence images, changes to CO$_2$CAS particle size and particle packing with different shear treatments is evident and reflect the particle size data determined in solution. As particle size was reduced between the UT1 and UT3 (Figure 2b and 2c, respectively) samples, the spacing (lighter areas) between large particles (darker areas) is decreased indicating closer particle packing in UT3 films. The stereofluorescence image for UT6 (Figure 2d) showed significant decrease in the particle size of CO$_2$-casein and resembled the image of the CaCAS film with increased homogeneity. The SEM images in Figure 3 show changes to the nanostructure, as particle size of CO$_2$CAS was reduced. The UT1 image (Figure 3a) shows well-defined, small circular particles. The UT3 film sample (Figure 3b) had less variation in the topography, and the particles were less well defined. The loss of definition in the UT3 sample could have been caused by closer packing of the protein particles. The UT6 image (Figure 3c) resolved small subunits indicating fracturing of the casein micelle with increasing shear treatment. The UT6 sample is similar to the CaCAS sample (Figure 3d). The changes to the microstructure and nanostructure observed in the microscopic images can be correlated to the changes in tensile properties and WVP of CO$_2$CAS films with decreasing particle size.

Tensile properties

Figure 4 shows the tensile properties for all CaCAS and CO$_2$CAS films as affected by glycerol content and particle size. Results are in agreement with previously reported values for CO$_2$CAS films plasticized with glycerol listed in Tomasula and others (1998). As expected, increasing plasticizer content significantly interrupted protein–protein interactions in the film matrices and decreased tensile strength and elastic modulus and increased elongation. For UT1- and UT3-film samples, when particle size was decreased from 126.4 μm to 111.8 μm, average overall tensile strength increased from 11.9 MPa to 13.7 MPa. Similarly, elastic modulus increased from 674.3 MPa to 950.5 MPa. Although the protein–glycerol mass ratios were the same for the UT1 and UT3 samples, more plasticizer would be required for the UT3 films to get the same tensile properties
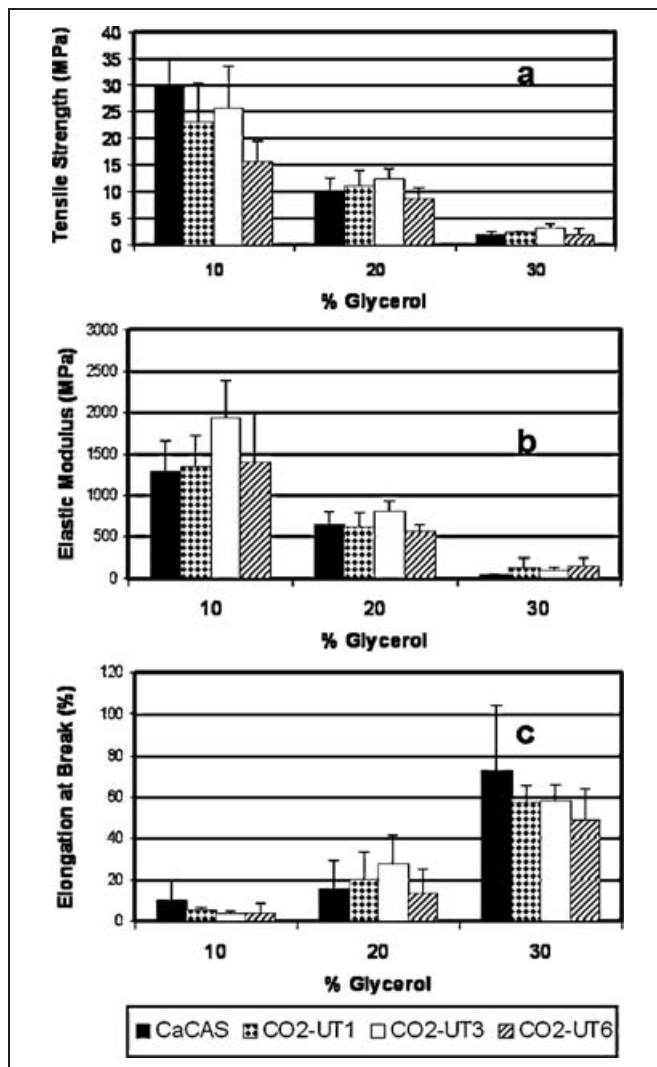

Figure 4—Tensile properties of calcium caseinate and CO$_2$-precipitated casein films with various levels of glycerol plasticizer: (a) tensile strength; (b) elastic modulus; (c) percent elongation at break.

as the UT1 films. This is because the decreased particle size in the CO$_2$CAS films treated with the Ultra-Turrax (IKA Works Inc.) 13500 rpm (UT3) allowed for closer protein particle packing and created more possibilities for protein–protein interactions. However, further reduction of particle size weakened CO$_2$CAS films. Tensile strength of the UT6 films decreased along with elastic modulus and elongation. The high shear of the UT6 treatment changed the micelle structure beyond particle size reduction as seen in the SEM images. Fracturing the micelle weakened the hydrophobic forces in comparison with those in the UT3 samples with intact micelles, leading to weaker films.

Water vapor permeability

Similar trends were seen with changes to WVP, as particle size of CO$_2$CAS was decreased in the films. Figure 5 shows average WVP results for all films. Averaged over all glycerol levels, the CaCAS films had the highest WVP at 5.09 cc mm/kPa m^2 h. This is consistent with Tomasula and others (1998), who found the CaCAS film had higher WVP compared with the CO$_2$CAS film. As particle size was reduced, average WVP of the CO$_2$CAS films decreased from 3.78 to 3.12 for the UT1 and UT3 films, respectively. As in the tensile properties, further reduction of the CO$_2$CAS particle size caused a negative effect of
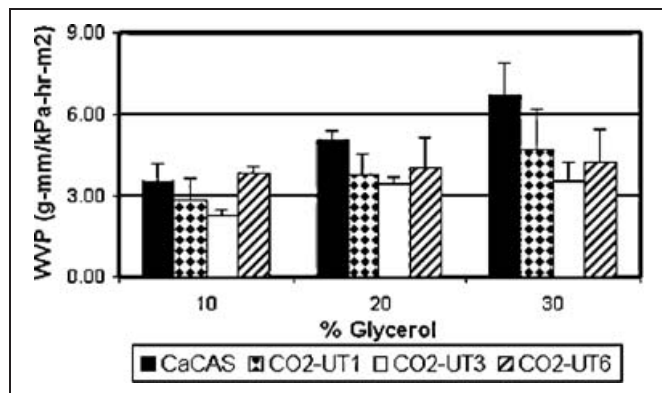
Figure 5 — Effect of decreasing particle size and glycerol amount on the water vapor permeability of CO₂-precipitated casein films.

Table 3 — Average thickness of calcium caseinate and CO₂-precipitated films plasticized with various levels of glycerol

Protein	Average thickness of films (mm)		
	10% Glycerol	20% Glycerol	30% Glycerol
Ca Caseinate	0.331 (0.079)[a]	0.301 (0.056)	0.280 (0.038)
CO₂CAS-UT6	0.334 (0.004)	0.306 (0.033)	0.203 (0.031)
CO₂CAS-UT3	0.236 (0.042)	0.229 (0.022)	0.175 (0.017)
CO₂CAS-UT1	0.282 (0.020)	0.272 (0.033)	0.261 (0.073)

[a]Standard deviations in parentheses.

film barrier properties with WVP increasing to 4.61 cc mm/kPa m^2 h for the UT6 sample. Looking at results for each of the individual plasticizer levels, the WVP of the UT3-treated CO₂CAS samples were the lowest of all film samples. It is assumed that the decrease of water vapor transmission in the UT3 films was caused by the denser packing of insoluble CO2-casein particles and the decrease in free volume. Average film thickness for all variables is shown in Table 3. For the same amount of plated solids and plasticizer ratios, UT3 film samples were thinnest, reflecting the tighter particle packing.

Conclusions

The gloss, haze, WVP, and tensile properties of high-pressure CO₂-precipitated casein films can be improved by reducing particle size of the protein. However, there is a limit. Further reduction with high shear treatment can cause fracturing of CO₂CAS micelles, subsequently weakening film. Because it is less soluble in water, CO₂CAS makes a better water barrier compared to many other hydrophilic agriculture hydrocolloids used to make edible films and coatings. Results of this study suggest films made from blends of reduced-particle-size CO₂CAS and the other more water-soluble hydrocolloids could have better water vapor barrier properties than the soluble hydrocolloids alone. By manipulating the free volume of the system and the protein–protein interactions, particle size reduction of CO₂CAS is an alternative procedure to the addition of plasticizer to affect film physical properties. This information could be used to design film and coating applications using CO₂CAS with desired appearance, barrier, or tensile properties.

References

ASTM. 1989. Standard test methods for specular gloss d523-89. In: Annual book of ASTM standards. Philadelphia, Pa.: American Society for Testing and Materials. P 32–5.

ASTM. 1995. Standard test methods for water vapor transmission of materials e96-95. In: Annual bood of ASTM standards. Philadelphia, Pa.: American Society for Testing and Materials. P 785–92.

ASTM. 1999. Standard test method for reflection haze of high-gloss surfaces d4039-93. In: Annual book of ASTM standards. Philadelphia, Pa.: American Society for Testing and Materials. P 1–2.

ASTM. 2001. Standard test method for tensile properties of thin plastic sheeting d882-01. In: Annual book of ASTM standards. Philadelphia, Pa.: American Society for Testing and Materials. P 162–70.

Brandenberg AH, Weller CL, Testin RF. 1993. Edible films and coatings from soy protein. J Food Sci 58:1086–9.

Cagri A, Ustunol Z, Ryser ET. 2004. Antimicrobial edible films and coatings. J Food Prot 67:833–48.

Cuq B, Guilbert S, Gontard N. 1998. Proteins as agricultural polymers for packaging production. Cereal Chem 75:1–9.

Driscoll DF. 2004. Examination of selection of light-scattering and light-obscuration acceptance criteria for lipid injectable emulsions. Pharmacopeial Forum 30:2–11.

Driscoll DF, Etzler F, Barber TA, Nehne J, Niemann W, Bistrian BR. 2002. The significance of particle/globule-sizing measurements in the safe use of intravenous lipid emulsions. J Dispersion Sci Technol 23:679–87.

Gennadios A, McHugh TH, Weller CL, Krochta JM. 1994. Edible coatings and films based on proteins. In: Krochta JM, Baldwin EA, Nisperos-Carriedo MO, editors. Edible coatings and films to improve food qualilty. Lancaster, Pa.: Technomic. P 201–78.

Hunter R. 1987. The measurement of appearance. 2nd ed. Norwich, N.J.: John Wiley and Sons, Inc. 432 p.

Lee SY, Dangaran KL, Guinard JX, Krochta JM. 2002. Consumer acceptance of whey-protein-coated as compared with shellac-coated chocolate. J Food Sci 67:2764–2769.

McHugh TH, Krochta JM. 1994. Dispersed phase particle size effects on water vapor permeability of whey protein-beeswax edible emulsion films. J Food Process Preserv 18:173–88.

McHugh TH, Avena-Bustillos RJ, Krochta JM. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. J Food Sci 58:899–903.

Perez-Gago M, Krochta JM. 2001. Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. J Agric Food Chem 49:996–1002.

Psomiadou E, Arvanitoyannis I, Yamamoto N. 1996. Edible films made from natural resources; microcrystalline cellulose (mcc), methylcellulose (mc) and corn starch and polyols— part 2. Carbohydrate Polymers 31:193–204.

Rogers CE. 1985. Permeation of gases and vapours in polymers. In: Comyn J, editor. Polymer permeability. London, England: Kluwer Academic Publishers. P 11–74.

Tomasula PM, Craig JC, Boswell RT, Cook RD, Kurantz MJ, Maxwell M. 1995. Preparation of casein using carbon dioxide. J Dairy Sci 78:506–14.

Tomasula PM, Parris N, Yee W, Coffin D. 1998. Properties of films made from CO₂-precipitated casein. J Agric Food Chem 11:4470–4.

E: Food Engineering & Physical Properties